Comparison of the Pulmonary Distribution and Efficacy of Antibodies Given to Mice by Intratracheal Instillation or Aerosol Inhalation

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Abstract I The respiratory tract is the portal of entry and target organ of many aerosolized toxins and infective agents, and there is increasing need for testing the efficacy of potential therapeutic agents delivered directly into the lungs. Intratracheal instillation and aerosol inhalation are the two methods most often used to introduce drugs, toxins, or infective agents into the respiratory tract of experimental animals. In this study we compared the distribution and efficacy of antibodies given to mice by aerosol inhalation or intratracheal instillation. We determined the pulmonary distribution of these antibodies by immunohistochemistry and observed the distribution and severity of pulmonary lesions that developed after exposure to aerosolized ricin. Although antibodies administered by either method prevented death, we found that instilled antibodies tended to concentrate around terminal airways and often failed to reach peripheral lung fields. Sometimes entire lung lobes were missed by the instillation route. Acute and chronic pulmonary lesions developed in the unprotected areas of instillation-treated lungs. In contrast, aerosolized antibodies covered all pulmonary surfaces and effectively prevented ricin-induced lesions throughout the lungs. Our findings suggest that the aerosol inhalation method may be preferable for evaluating the efficacy of therapeutic agents in the respiratory tract because of the failure of instilled agents to reach and protect peripheral alveoli.

The respiratory tract is the portal of entry and target organ of many aerosolized toxins and infective agents. The respiratory tract is also the preferred route of administration for a variety of therapeutic agents and antimicrobials (1–3). There is increasing need to test the efficacy and toxicity of potential therapeutic agents delivered directly into the lungs of experimental animals. Recently, novel therapeutic agents such as surfactants (4) and deoxyribonuclease (5) have been given via the respiratory tract. Aerosolized antibodies have promise for treating viral infections in the lung (6), and innovative combinations such as liposome-encapsulated antibodies (7) or bispecific antibodies (8) may prove useful in the future.

Therapeutic agents are often more effective in treating primary lung disease when administered directly into the respiratory tract than when given by other routes. For example, pentamidine isethionate, when it was originally administered to rats by intramuscular injection, failed to clear *Pneumocystis carinii* infections (9). However, the same drug was later proven to be effective in rats when administered as an aerosol (10); aerosolized pentamidine has since become the drug of choice for treating *Pneumocystis carinii* pneumonia. Similarly, aerosolized (but not parenteral) interferon gamma administration reduces allergic responses and improves airway functions in mice with experimentally induced pulmonary hypersensitivity (11). Thus direct treatment of the respiratory tract can deliver effective doses of expensive, poorly soluble, or systemically toxic compounds to the pulmonary parenchyma.
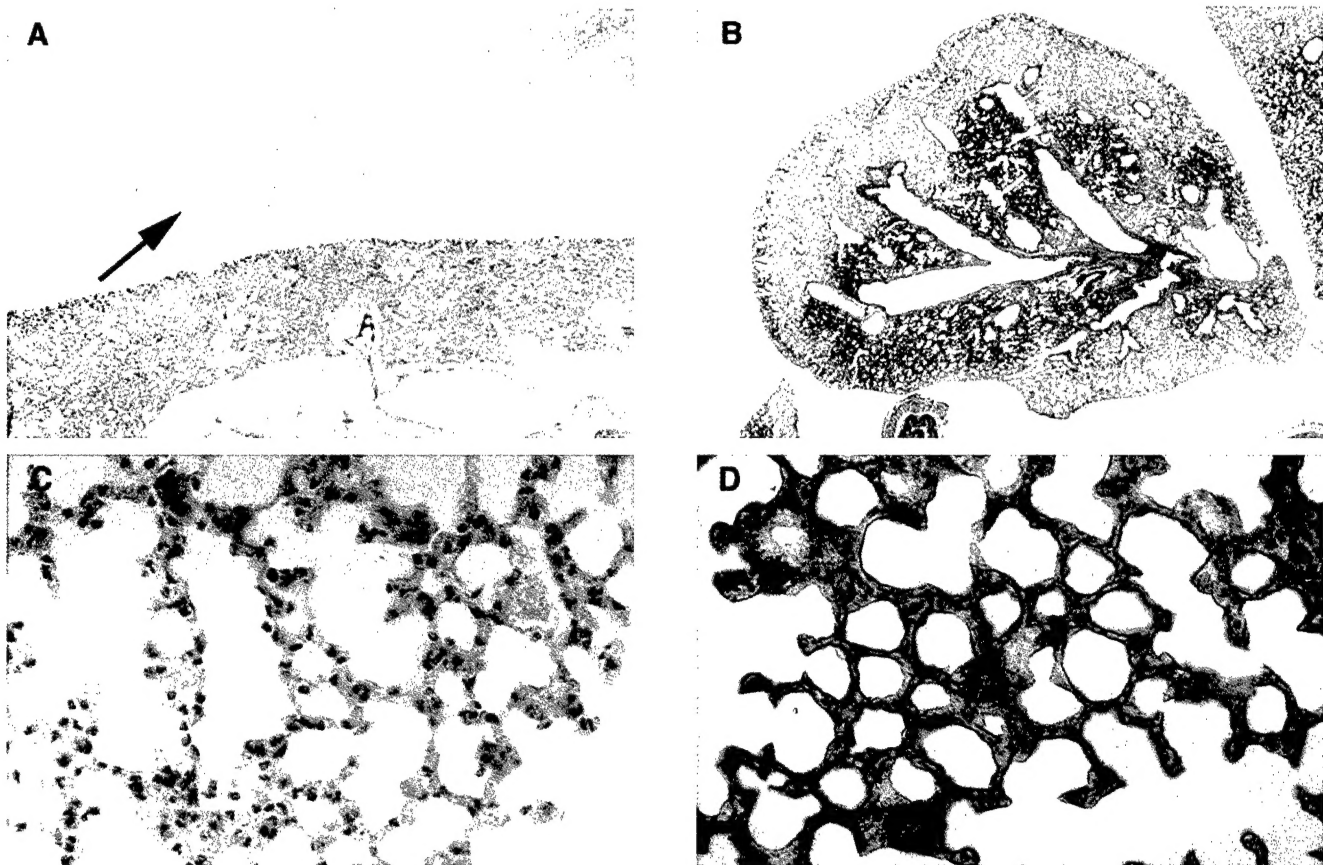
Drugs, toxins, or infective agents are usually introduced into the respiratory tract of experimental animals by either intratracheal instillation or aerosol inhalation. In the first method a prepared suspension or solution is instilled directly into the tracheal lumen, whereas in the second an aerosol is generated by a nebulizer and inhaled by the animal. Generally the aerosol inhalation method is preferred as the more "natural" or "physiologic" exposure route. However, intratracheal instillation is a widely used alternative for several reasons. Instillation is inexpensive, requires minimal equipment, and is easily learned, whereas aerosol inhalation requires expensive generating and monitoring equipment and complex technical support. An important advantage of the aerosol exposure method was reported in studies indicating that the pulmonary distribution of dust particles is more homogeneous when given by aerosol than when given as an aqueous suspension by intra-

Figure 1. Immunocytochemical detection of ricin-specific goat antibodies in CD-1 mouse lungs at 1 h after treatment. Antibodies were administered by either intratracheal instillation (**A–C**) or aerosol inhalation (**D**) and were detected by a biotinylated anti-goat IgG and alkaline phosphatase-labeled streptavidin system. The distribution of intratracheally instilled antibodies was uneven: in panel **A** there is complete antibody coverage of the right middle lobe of the lung (bottom) but only minimal coverage in the adjacent accessory lobe (arrow). Instilled antibodies tended to concentrate around terminal airways and surrounding alveoli and were usually not detectable in the peripheral alveoli (**B**). At higher magnification ricin-specific antibodies are undetectable in the peripheral alveoli of this instillation-treated lung (**C**). In contrast, staining for antibodies was consistently present on peripheral alveolar septa of all lung lobes in aerosol-treated mice (**D**). Sections immunostained with Histomark Red (**A** and **B**) or diaminobenzidine (**C** and **D**) and counterstained with hematoxylin. Original magnification 4x for **A**; 2x for **B**; and 40x for **C** and **D**.

tracheal instillation (12, 13). However, the distribution and therapeutic efficacy of antibodies delivered by these same two methods have not been reported.

In this study we used immunohistochemistry techniques to compare the pulmonary distribution of protective doses of ricin-specific antibodies administered to mice by either aerosol inhalation or intratracheal instillation. We then compared the efficacy of ricin-specific antibody treatments by these two methods, evaluating the distribution and severity of pulmonary lesions that developed in mice after exposure to aerosolized ricin toxin.

Materials and Methods

Animals: Male CD-1 mice (Charles River Laboratories, Wilmington, Mass.) weighing 26 to 28 g were studied. The mice were housed in an AAALAC, International-accredited facility in animal rooms maintained at 20 to 22°C, with a relative humidity of 20 to 50% and a 12/12-h light/dark cycle. The mice were kept in polycarbonate shoebox-type cages with filter cap tops and with pelletized organic cellu-

lose fiber bedding (Cellu-Dri; Shephard Specialty Papers, Inc., Kalamazoo, Mich.). The mice were fed a standard commercial diet (NIH 0-7 Rat and Mouse Feed; Zeigler Bros., Gardners, Pa.) and water ad libitum. Mice were humanely killed by intraperitoneal injection of sodium pentobarbital.

Experimental design: This report combines the results from a series of experiments comparing the distribution and efficacy of antibodies administered by intratracheal instillation and aerosol inhalation. The antibody doses chosen for these experiments were based on preliminary studies in which we determined the doses of inhaled (24 μg) and instilled (240 μg) ricin-specific antibodies that prevented death in mice exposed to otherwise lethal doses of aerosolized ricin (unpublished data). The types of treatments, exposure methods (intratracheal instillation or aerosol inhalation), ricin challenge, and number of mice in each group are summarized in Table 1. Untreated control mice and mice treated with nonspecific goat immunoglobulin G (IgG) (n = 48 mice) that had not been taken at random and euthanized by postexposure day 2 served to
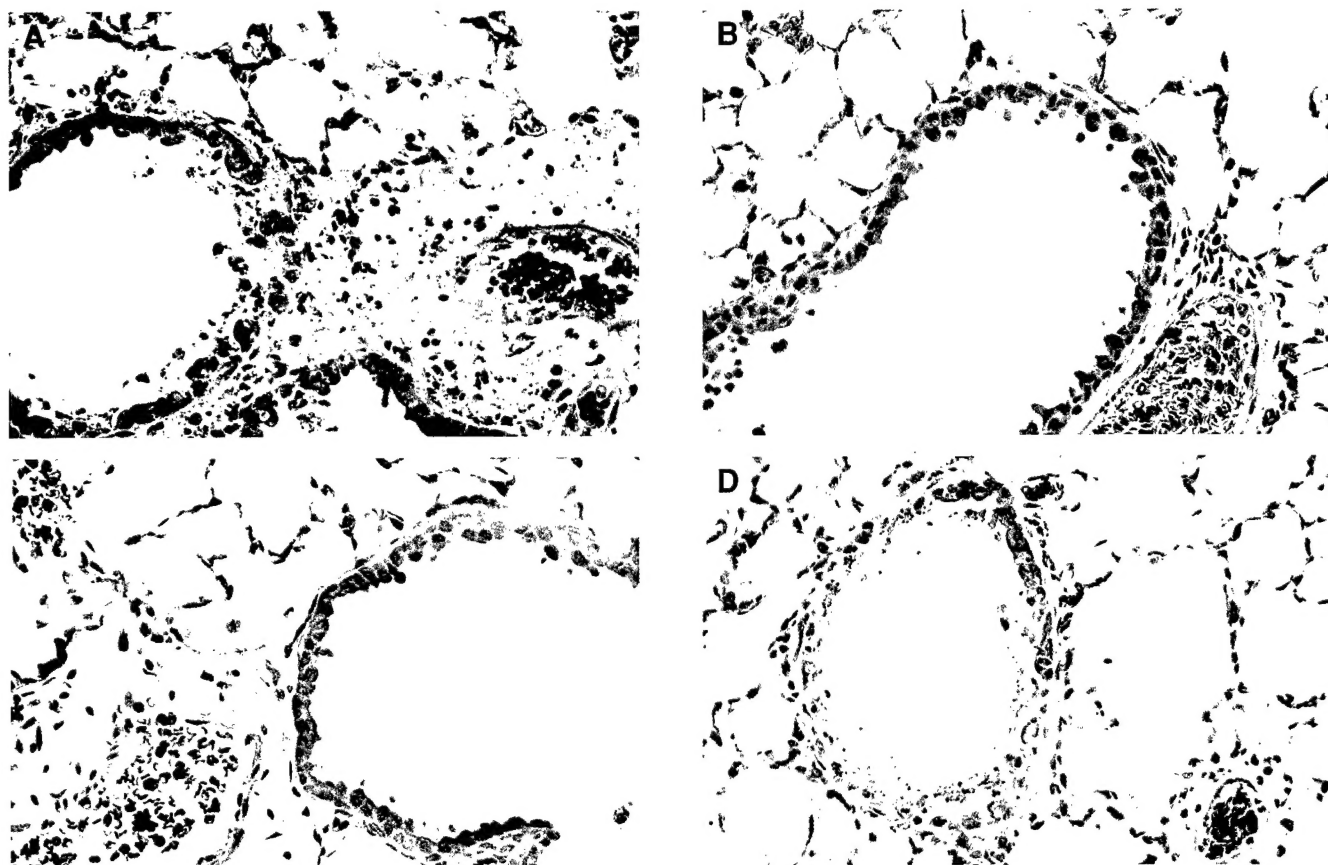
Figure 2. Histologic lesions in CD-1 mouse lungs at 48 h after exposure to ricin toxin. In untreated control mice there is diffuse necrosis of airway and alveolar epithelium, as well as diffuse perivascular and alveolar edema and inflammation (**A**). Lesions are mild or absent in the antibody-treated areas of the lung in both aerosol- (**B**) and instillation- (**C**) treated mice. However, the uneven distribution of antibodies in instillation-treated lungs allowed lesions to develop in untreated areas. Panel **C** shows the essentially normal appearance of the terminal airways and alveoli in the right middle lung lobe. In contrast, panel **D** shows the extensive epithelial necrosis, inflammation, and edema in the unprotected right accessory lobe of the same instillation-treated lung. Original magnification was 40x for **A–D**.

verify that an LC99 toxin exposure had occurred. By day 4 all mice had died from the ricin exposure. No tissues were collected from these untreated (n = 16) and nonspecific IgG instillation- (n = 14) and aerosol- (n = 18) treated mice. The higher dose of nonspecific antibodies (54 μg) given by aerosol was not expected to affect survival rates after ricin exposure (in fact all mice died) but was used to improve the immunohistochemical detection of aerosolized antibody distribution in the lung.

We first determined the distribution of nonspecific (goat IgG) and ricin-specific antibodies administered by intratracheal instillation. Mice in groups 1 and 2 received 300 μg of nonspecific goat IgG; groups 3, 4, and 5 received 300 μg of ricin-specific IgG. The mice in groups 6 through 10 were treated with aerosolized antibodies. Mice in groups 6 and 7 received 54 μg of nonspecific goat IgG; mice in groups 8, 9, and 10 received 24 μg of ricin-specific antibodies. Pulmonary distribution of antibodies was determined by immunohistochemistry in the mice that were killed within 2 h of treatment (groups 1, 3, 6, and 8). These lungs were immersion-fixed in formalin.

We then evaluated the efficacy of both treatment methods

for preventing acute and chronic ricin-induced lesions in the lungs. Some mice were given either nonspecific goat IgG or ricin-specific antibodies by intratracheal instillation (groups 1 through 5) before exposure to aerosolized ricin toxin. Other mice were pretreated with aerosolized nonspecific IgG or ricin-specific antibodies (groups 6 through 10). The aerosol-treated mice were part of a larger study that examined the dose-response effects of aerosolized antibodies in ricin-exposed lungs. One hour after treating mice with instilled or aerosolized antibodies, we exposed all mice to a lethal dose of aerosolized ricin. Aerosol-treated mice received a calculated ricin dose of 2.8 to 3.1 μg/L of air, and instillation-treated mice received 11.8 μg/L of air. We completed histopathologic findings in lungs taken from three to six mice selected randomly at 0, 2, and 14 days after ricin exposure. We also examined the lungs taken from all surviving mice in each group at postexposure day 14. Lungs intended for histologic evaluation were first fully inflated by intratracheal infusion of formalin, then completely fixed by immersion in formalin. In contrast, lungs intended for immunohistochemical determination of antibody distribution were fixed by immersion only, because infusion of fixa-

Table 1. Treatment and exposure groups

Group no.	Treatment method	Treatment	Antibody dose (µg)	Ricin (µg/L)	Postexposure days	No. of mice
1	IT	Goat IgG	300	11.8	0	4
3	IT	Goat IgG	300	11.8	2	6
2	IT	Ricin-specific IgG	300	11.8	0	4
4	IT	Ricin-specific IgG	300	11.8	2	6
5	IT	Ricin-specific IgG	300	11.8	14	6
6	AER	Goat IgG	54	2.8–3.1	0	5
8	AER	Goat IgG	54	2.8–3.1	2	3
7	AER	Ricin-specific IgG	24	2.8–3.1	0	5
9	AER	Ricin-specific IgG	24	2.8–3.1	2	3
10	AER	Ricin-specific IgG	24	2.8–3.1	14	8

IT = instillation, AER = aerosol

Table 2. Extent of pulmonary lesions at 2 days after exposure to ricin

Treatment group	Bronchiolar necrosis*	Alveolar necrosis[†]
Intratracheal specific Ab	36	40
Aerosolized specific Ab	1	3
Intratracheal nonspecific Ab	93	96
Aerosolized nonspecific Ab	95	96

*Bronchiolar necrosis: percentage of airway cross sections with >10% epithelial necrosis
[†]Alveolar necrosis: percentage of alveoli with >4 necrotic cells in a 40x field
Ab = antibody

tive would have altered antibody distribution. All sections for histologic evaluation were randomized and scored blindly by the pathologist, and the average scores were recorded. The immunohistochemical localization of antibodies was determined on multiple sections of each mouse lung, with each section containing at least four of the five lung lobes.

Preparation of antibodies: Affinity-purified goat anti-ricin IgG was prepared as described (14). The affinity-purified IgG was then dissolved in endotoxin-free saline at 14 mg/ml and passed over Pyrobind ST endotoxin-removal cartridges (Sepracor, Marlborough, Mass.) to remove bacterial endotoxins. Residual endotoxin in the antibody preparation was 0.04 EU/mg of IgG for the anti-ricin antibody and 0.01 EU/mg of IgG for the control antibody (QCL-1000 Quantitative Chromogenic LAL; BioWhittaker, Walkersville, Md.).

Ricin toxin: A 5-mg/ml stock solution of *Ricinus communis* agglutinin II (Vector Laboratories, Burlingame, Calif.) was diluted to desired concentrations in endotoxin-free distilled water.

Aerosol generation: Ricin-specific antibodies and ricin toxin were diluted as required in 8 ml of phosphate-buffered saline. We used identical reagent concentrations (ricin = 1 mg/ml, antibodies = 14 mg/ml) for all exposures, with variations in inhaled dosage produced by altering exposure time. Instillation-treated mice (groups 1 through 5) were given aerosolized ricin (approx. 12 µg/L) in a nose-only exposure system (15). Aerosol-treated mice in groups 6 through 10 were exposed to small-particle aerosols of antibody or ricin (approx. 3 µg/L) in a whole-body exposure system set up inside a class-III hood. These aerosols were generated with a Collison nebulizer (16) driven by compressed air at 26 lb/in² (gauge). This nebulizer produced an aerosol with a flow rate of 7.5 L/min and a mass median aerosol diameter of 1.2 µm. This aerosol was mixed with 12 L/min of secondary air before it entered the exposure

system for a total system flow rate of 19.5 L/min. The aerosol was sampled continuously during exposure by an all-glass impinger, and protein concentrations in the impinger samples were determined by the micro BCA protein assay kit (Pierce, Rockford, Ill.). Installation-treated mice were given aerosolized ricin with a nose-only exposure apparatus. Aerosol concentrations (µg/L) were calculated, and the inhaled antibody dose was estimated by Guyton's formula for minute volume calculations. Each mouse in groups 6 and 7 received a calculated dose of 54 µg of nonspecific goat IgG, and each mouse in groups 8 through 10 received a calculated dose of 24 µg of ricin-specific goat IgG.

Intratracheal instillation: We used Saffioti's method (17) for intratracheal instillations. Briefly, mice were anesthetized by intraperitoneal injection of 50 to 75 mg of sodium pentobarbital/kg of body weight and were positioned on a slanted board (30° from vertical). A bent, blunted 23-gauge needle attached to a 1-ml tuberculin syringe was inserted under the glottis into the trachea, and 200 µl of the antibody solution was infused directly into the trachea. Each mouse in groups 3 through 5 received 300 µg of ricin-specific goat IgG by intratracheal instillation.

Immunohistochemistry: We determined the distribution of ricin-specific antibodies by immunohistochemistry on lung tissues collected approximately 1 h after intratracheal or aerosol treatment with antibodies (groups 1, 3, 6, and 8). Tissue sections were processed by alkaline phosphatase-labeled streptavidin or avidin-biotin peroxidase complex detection systems. These tissues were fixed by immersion in neutral buffered 10% formalin. Tissues were embedded in paraffin, sectioned at 5 to 6 µm, and mounted on positively charged glass slides (SuperfrostPlus; Fisher Scientific, Pittsburg, Pa.), deparaffinized, rehydrated, then blocked in 2% normal rabbit serum in phosphate-buffered saline for 20 min at room temperature in a humidified chamber. Nonspecific peroxidase was blocked with a 1% hydrogen peroxide in methanol solution. After the blocking serum was drained, goat anti-ricin IgG was detected in the lungs by a biotinylated rabbit anti-goat secondary antibody (Vector Laboratories) diluted 1:200 in phosphate-buffered saline with 0.2% Tween 20 and 5% normal mouse serum. After another Tween 20 wash, sections were incubated for 30 min with alkaline phosphatase-labeled streptavidin (GIBCO BRL, Gaithersburg, Md.), diluted 1:50 in Tween 20 or avidin-biotin peroxidase complex (Vector Laboratories). The chromogenic substrate for alkaline phosphatase was
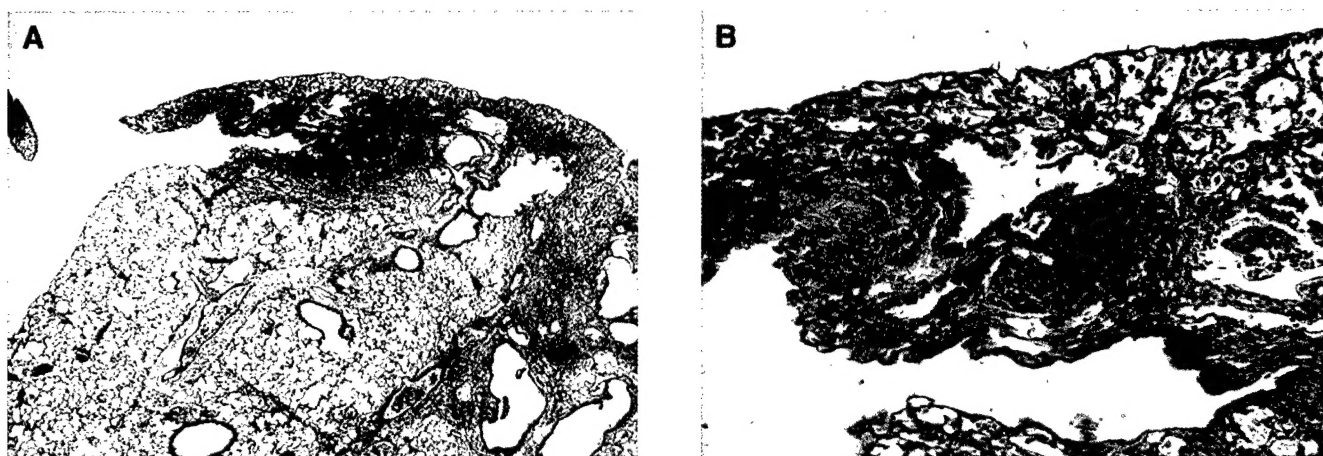
Figure 3. Photomicrographs of sections of the peripheral lung lesions in instillation-treated mice at 14 days after exposure. After full inflation of the lungs by the infusion of fixative, areas of atelectasis and inflammation were still evident in peripheral lung fields (**A**). These chronic lesions were characterized by lymphocytic and histiocytic inflammation superimposed on areas of alveolar collapse and septal fibrosis (**B**). Original magnification was 10x for **A** and 40x for **B**.

HistoMark™ Red (Kirkegaard & Perry, Gaithersburg, Md.), and the peroxidase substrate was diaminobenzidine. After color development was completed, sections were washed in distilled water and counterstained with Mayer's hematoxylin (Poly Scientific, Bay Shore, N.Y.). Negative controls included deletion of the biotinylated anti-goat IgG antibodies and application of the biotinylated anti-goat IgG antisera to untreated mouse lungs.

Histologic examination: Ricin-exposed mice were euthanized by intraperitoneal injection of pentobarbital at 2 and 14 days after exposure. The heart and lungs were excised together from the carcass. At necropsy these ricin-exposed lungs were first fully inflated by infusion of fixative, then immersion-fixed with neutral-buffered 10% formalin. Tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Each high-power field (x400 magnification) was examined for necrosis in airways and alveoli. Fields were scored as positive for severe alveolar necrosis if >5 necrotic cells (characterized by pyknosis, karyorrhexis, and cytoplasmic fragmentation) were detected in any alveolus within the high-power field. Bronchiolar necrosis was graded as severe if >75% of airway epithelium within a given bronchiole was denuded or necrotic. Essentially, normal airways contained <1% necrotic bronchiolar epithelial cells. Other degrees of airway necrosis in each high-power field were graded as intermediate.

Results

Antibody distribution: Pulmonary distribution of antibodies administered by intratracheal instillation was highly variable within and between lung lobes. In some mice, staining was absent in entire lobes (most often the right accessory lobe–Figure 1A) and in one mouse was present only in the left lung. Staining of intratracheally instilled antibodies was most intense in the terminal airways and surrounding alveoli (Figure 1B). Moreover, staining usually did not extend to the peripheral alveoli in the instillation-treated lungs (Figure 1C).

In contrast, aerosolized antibodies covered all lung lobes in every mouse. Although the overall staining intensity of aerosolized antibodies was weak, this reduced staining intensity was consistent with the lesser amounts of antibody delivered by the aerosol route. In the aerosol-treated mice the general pattern of antibody distribution in treated lobes, although of weaker intensity, was otherwise similar to that seen in the treated lung lobes of the instilled mice (Figure 1B). In both instances the most intense antibody staining was typically located around terminal airways. However, in marked contrast to the instillation-treated mice, antibody coverage extended into the peripheral alveoli of all lobes in the aerosol-treated mice (Figure 1D).

Histopathologic findings: Diffuse, severe pulmonary lesions were present in all ricin-challenged mice that were given nonspecific goat IgG by either method. In these lungs there was diffuse airway and alveolar epithelial necrosis, with widespread perivascular/peribronchiolar and alveolar inflammation and edema (Figure 2A). By 2 days after exposure, ricin toxin caused marked to severe airway and alveolar epithelial necrosis in all lung lobes of these mice. Alveolar necrosis and severe bronchiolar necrosis were observed in well over 90% of all 40x high-power fields in these lungs (Table 2). Epithelial necrosis was most severe in the trachea and proximal bronchi. The loss of terminal airway epithelium resulted in plugging of the respiratory bronchioles and alveolar ducts with necrotic cell debris. There was marked leakage of fibrin and proteinaceous fluid into the alveoli with pseudomembrane formation. Alveolar septa were thickened by a combination of neutrophils and edema fluid, and most alveoli contained necrotic cell debris and protein fluid (alveolar edema). Alveolar macrophages were absent or necrotic. There also was diffuse peritracheal, periesophageal, and interstitial inflammation with edema. Vessel walls were sometimes disrupted by the many transmigrating neutrophils, and lymphatics were dilated and filled with proteinaceous fluid. Inflammation and necrosis tended to be most severe in focally extensive pleural and

subpleural locations. The tracheobronchial lymph nodes contained many sinusoidal macrophages distended with engulfed cell debris. Lymphocytolysis was also apparent in the lymphoid follicles. All mice in the untreated and non-specific IgG-treated groups died within 4 days of exposure to aerosolized ricin. These unprotected mice were used to verify that LC99 toxin exposure had been achieved, and no tissues were collected or examined from these animals.

In contrast, ricin-specific antibodies administered by either method provided substantial protection from ricin-induced lesions in the lung (Figures 2B and 2C). However, lesions were still present multifocally in the instillation-treated mice, and the distribution and severity of these pulmonary lesions mirrored the uneven distribution of the anti-ricin antibodies given by intratracheal instillation (Figure 2D).

Necrosis of pneumocytes (types I and II) was observed in all lungs in this group; however, the ricin-specific antibody reduced the severity and extent of epithelial damage. These lungs contained some perivascular edema and inflammation, and alveolar necrosis was detected in 40% of high-power fields. Severe bronchiolar necrosis affected 36% of small airways. The severity of lesions varied markedly between lung lobes in individual animals, with the right accessory lobe particularly susceptible to severe damage (Figure 2D). In affected areas there was damage to the integrity of the alveolar septa, as evidenced by fibrin and proteinaceous fluid in the alveolar spaces and variable numbers of intra-alveolar histiocytes and neutrophils. Airway epithelial necrosis was most severe in the distal airways; debris and inflammatory cells tended to accumulate at the bronchioloalveolar junction. Mediastinal edema, inflammation, and necrosis were absent in specific anti-body-treated mice at 48 h after exposure. Morphologic evidence of necrosis of ciliated epithelium was generally minimal or absent in the larger airways and nasal mucosa.

At 2 days after exposure the protective effects of the aerosolized ricin-specific antibodies were even more pronounced. Alveolar and bronchiolar necrosis was present in <5% of the high-power fields, and we found lesions in the proximal portion of the trachea of only one mouse. In this mouse there were areas where ciliated epithelial cells were absent, and the tracheal epithelium was multilayered and flat (squamous metaplasia). The distal portion of the trachea and bronchial/bronchiolar epithelium was normal. Lungs of aerosol-treated mice were essentially normal 14 days after ricin exposure.

In the instillation-treated mice at day 14 after exposure the nasal, tracheal, bronchial, and bronchiolar epithelium also returned to normal. However, multiple foci of chronic inflammation and atelectasis were still evident in peripheral lung fields (Figure 3A). These chronic subpleural lesions appeared as purple-red depressions in the lung surface and were often located at the margins of lung lobes. These lesions were grossly visible even after full inflation of the lung with fixative and microscopically were composed of lymphoplasmacytic and histiocytic infiltrates within fibrous walls and lumens of affected alveoli (Figure 3B). These lesions generally affected <5% of the pulmonary parenchyma.

Discussion

Ricin-specific antibodies administered to mice by either intratracheal instillation or aerosol inhalation reduced pulmonary damage and prevented death from otherwise lethal doses of inhaled ricin toxin. However, we found important differences in the distribution and protective effects of antibodies when they were given by these two methods. Although these differences may not be important in some toxicologic and carcinogenesis studies, they could possibly have a major impact on the results of drug efficacy trials against some pulmonary pathogens.

Previous reports suggested that intratracheal instillation is equivalent or even superior to aerosol inhalation in some applications (18–22). Intratracheal instillation is clearly more effective in establishing experimental pulmonary infections than is aerosol exposure (20–22). Our finding that instilled antibodies were mostly concentrated around terminal airways may help explain the increased infectivity of instilled inocula. When instilled, infective organisms may be more concentrated around terminal airways, allowing the massed inoculum to overwhelm the local pulmonary defenses. Instillation also avoids the desiccation and inactivation of infective agents that might occur during nebulization.

Instillation has been effectively used in testing the pulmonary toxicity of a variety of materials (18, 19). Intratracheal instillation permits precise control of the dose given to each animal, is not influenced by particle size, and reduces the hazards and decontamination problems associated with handling toxic materials. In contrast, inhaled doses may vary more than 20% (23), with the size and distribution of an inhaled dose influenced by minute ventilation times, particle size of the aerosol, breathing pattern of the animal, and dimensions of the airways and alveoli (13). When whole-body aerosol exposure methods are used, dosage variations can result from percutaneous absorption or ingestion during grooming. Also, because rodents are obligate nose breathers, most of a large-particle aerosol may be trapped in the upper respiratory tract (24).

We compared the pulmonary distribution of antibodies given by intratracheal instillation and aerosol inhalation with immunohistochemistry staining methods and found that the distribution of intratracheally instilled antibodies was not homogeneous. Instilled antibodies were concentrated around terminal airways; they rarely penetrated to the peripheral alveoli and occasionally were absent from entire lung lobes. In contrast to the uneven pulmonary distribution of antibodies administered by instillation, aerosolized antibodies were more uniformly distributed and were consistently detectable in peripheral alveoli. The overall weaker staining for aerosolized antibodies that we observed was consistent with the 10-fold lower dosage of antibodies administered by aerosol. These method-dependent differences in antibody distribution are similar to those reported for instilled and aerosolized dusts in rats (12, 13). In those studies the distribution of dust obtained by instillation was shown to be less homogeneous than that obtained by inhalation, and penetration to the periphery was minimal.

Although it is possible that we could have improved coverage of peripheral lung fields by increasing the instilled

volume of fluid, it is likely that trapped air in peripheral alveoli would have prevented complete coverage. Pritchard (12) instilled volumes ranging from 100 to 750 µl into 350-g rats and found that the distribution of instilled dust particles was not improved by increasing the volume of suspension above 500 µl or increasing the concentration of the suspension. Moreover, fatality rates increased with larger volumes. Including about 300 µl of air in the dosing syringe and using this extra air to push suspensions deeper into the lungs resulted in better coverage of peripheral areas. However, even with this modification, coverage was less homogenous than that obtained by aerosol administration.

We exposed antibody-treated mice to aerosolized ricin toxin to assess the efficacy of antibodies administered by either instillation or inhalation. We believe that the variations in distribution of protective antibodies were principally responsible for the observed differences in the severity and distribution of lesions that developed after exposure to aerosolized ricin toxin. We cannot exclude the possibility that some of the differences we observed were due to the different amounts of ricin that the aerosol and instillation treatment groups received (2.8 to 3.1 and 11.8 µg/L of air respectively, 10-min exposure); however, it is noteworthy that these concentrations are within established LC99 values, and 100% lethality was observed in all untreated and nonspecific antibody-treated control groups. Furthermore we did not observe any differences in pathologic findings in the untreated control mice at early time points compared with mice of the treatment groups. The severe pulmonary lesions that were present in untreated control mice at 2 days after exposure were virtually identical in the installation- and aerosol-treated groups. However, major differences were evident in the IgG-treated groups at 2 days after exposure. The acute necrotizing bronchiolar and alveolar lesions in untreated control mice were milder and less diffuse in the installation-treated mice and were essentially absent in the aerosol-treated mice (Table 2). Although instilled ricin-specific antibodies markedly reduced the severity of lesions in the upper airways (trachea and bronchi) and mediastinum, the protective effects of pretreatment were less uniform in the lower airways and alveoli. Pulmonary lesions developed principally in the unprotected lobes and peripheral lung fields of intratracheal instillation-treated mice.

Similarly the chronic subpleural lesions at the margins of most lung lobes most likely resulted from the lack of antibody coverage in these areas, as well as the less efficient clearance of necrotic debris from peripheral alveoli. The persistence of necrotic debris and inflammatory cells (and possibly ricin) within peripheral alveoli may have contributed to the septal fibrosis, lymphoplasmacytic and histiocytic nodules that developed in these peripheral locations. Although these chronic lesions in instillation-treated mice apparently did not involve sufficient lung parenchyma to result in clinical signs of disease, they would be likely to persist for an extended time and could have important implications. The failure of instilled antibodies to reach and protect peripheral areas could have a substantial impact on antimicrobial efficacy trials. We speculate that pathogenic organisms might survive in untreated peripheral areas of the lung after instillation of antibodies or antimicrobials, and these surviving pathogens could cause recrudescent infections. In contrast, aerosolized antibodies covered all areas of the lung and prevented acute and chronic peripheral lesions attributable to ricin.

We found that the distribution and efficacy of antibodies administered by aerosol was superior to instilled antibodies. Even though intratracheal instillation is a simple and inexpensive method with proven advantages for some applications, the incomplete coverage and resulting pulmonary lesions it allows to develop suggest that it may not be suitable for evaluating the efficacy of therapeutic agents against pulmonary pathogens. Aerosolized treatments would be preferable in therapeutic trials because the failure of instilled agents to reach and protect peripheral areas could allow pathogens in these areas to survive and later cause recrudescent infections.

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13. ABSTRACT (Maximum 200 words)
The respiratory tract is the portal of entry and target organ of many aerosolized toxins and infective agents, and there is increasing need for testing the efficacy of potential therapeutic agents delivered directly into the lungs. Intratracheal instillation and aerosol inhalation are the two methods most often used to introduce drugs, toxins, or infective agents into the respiratory tract of experimental animals. In this study we compared the distribution and efficacy of antibodies given to mice by aerosol inhalation or intratracheal instillation. We determined the pulmonary distribution of these antibodies by immunohistochemistry and observed the distribution and severity of pulmonary lesions that developed after exposure to aerosolized ricin. Although antibodies administered by either method prevented death, we found that instilled antibodies tended to concentrate around terminal airways and often failed to reach peripheral lung fields. Sometimes entire lung lobes were missed by the instillation route. Acute and chronic pulmonary lesions developed in the unprotected areas of instillation-treated lungs. In contrast, aerosolized antibodies covered all pulmonary surfaces and effectively prevented ricin-induced lesions throughout the lungs. Our findings suggest that the aerosol inhalation method may be preferable for evaluating the efficacy of therapeutic agents in the respiratory tract because of the failure of instilled agents to reach and protect peripheral alveoli.

14. SUBJECT TERMS
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